

## What is claimed is:

- 1. A cloning system comprising:
  - (a) a first arm having a first selectable marker and a first cyclization element; and
  - (b) a second arm having a second selectable marker and a second cyclication element,

wherein at least one arm further comprises an origin of replication.

- 2. The cloning system of claim 1, wherein each arm further comprises a rare restriction endonuclease recognition site.
- The cloning system of claim 1, wherein each arm further comprises a polylinker.
- 4. The cloning system of claim 1, wherein said first cyclization element is a nucleic acid comprising a first LOX site, and said second cyclization element is a nucleic acid comprising a second LOX site.
- 5. The cloning system of claim 1 wherein:
  - the first arm further comprises a first nucleic acid homologous to the 5' terminus of a target nucleic acid; and
  - (b) the second arm further comprises a second nucleic acid homologous to the 3' terminus of the target nucleic acid.
- 6. A composition comprising said cloning system of claim 1 and a target sequence.
- 7. The composition of claim 6, wherein said target sequence is a nucleic acid of a virus.
- 8. The composition of claim 7, wherein said virus is a DNA virus.
- 9. The composition of claim 8, wherein said DNA virus is selected from the group consisting of adenovirus, adeno-associated virus, pox virus, papova virus and herpesvirus.



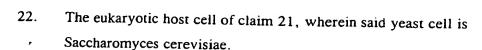
- 10. The composition of claim 7, wherein said virus is an RNA virus.
- 11. The composition of claim 10, wherein said RNA virus is a retrovirus.
- 12. The composition of claim 11, wherein said retrovirus is a lentivirus.
- 13. The composition of claim 12, wherein said lentivirus is human immunodeficiency virus.
- 14. A vector comprising:
  - (a) a yeast selectable marker;
  - (b) a bacterial selectable marker;
  - (c) a telomere;
  - (d) a centromere;
  - (e) a bacterial replication element;
  - (f) a yeast replication element; and
  - (g) at least one rare restriction endonuclease recognition site.
- 15. The vector according to claim 14, comprising at least one unique restriction endonuclease recognition site.
- 16. The vector according to claim 14, comprising a polylinker.
- 17. The vector of claim 14, comprising a first nucleic acid homologous to the 5' terminus of a target nucleic acid, and a second nucleic acid homologous to the 3' terminus of said target nucleic acid.
- 18. The vector of claim 14, further comprising a target nucleic acid.
- 19. The vector of claim 18, wherein said target nucleic acid is a nucleic acid sequence of a virus.

SUB B3 20. A eukaryotic host cell comprising said cloning system of claim 1.

21. The eukaryotic host cell of claim 20, wherein said eukaryotic host cell is a yeast cell.



- 36. The method of claim 35, wherein at least one arm further comprises an origin of replication.
- 37. The method of claim 35, wherein each arm further comprises a rare restriction endonuclease recognition site.
- 38. The method of claim 35, wherein said first cyclization element is a nucleic acid comprising a first LoxP site, and the second cyclization element is a nucleic acid comprising a second LoxP site.
- 39. The method of claim 35, wherein homologous recombination occurs in a yeast cell.
- 40. The method of claim 35, further comprising the step of circularizing said vector containing said target nucleic acid.
- 41. The method of claim 38, wherein said vector is circularized by contacting said first and said second LoxP sites with Cre, thereby producing a circularized recombinant vector by site-specific recombination.
- 42. The method of claim 38, wherein said vector is circularized in bacteria.
- 43. The method of claim 35, further comprising introducing said vector containing said target nucleic acid in a bacterium to propagate said vector.
- 44. A method of producing a recombinant nucleic acid comprising:
  - (a) contacting:
    - (i) a target nucleic acid; and
    - (ii) a vector comprising in operable linkage:
      - (1) a yeast selectable marker;
      - (2) a bacterial selectable marker;
      - (3) a telomere:
      - (4) a centromere;
      - (5) a yeast replication element:
      - (6) a bacterial replication element;



- 23. A cell comprising the vector of claim 14.
- 24. The cell of claim 23, which is a eukaryotic cell.
- 25. The cell of claim 24, wherein said eukaryotic cell is a yeast cell.
- 26. The cell of claim 25, wherein said yeast cell is Saccharomyces cerevisiae.
- 27. A cell comprising the composition of claim 6.
- 28. The cell of claim 27 which is a eukaryotic cell.
- 29. The cell of claim 28, wherein said eukaryotic cell is a yeast cell.
- 30. The cell of claim 29, wherein said yeast cell is Saccharomyces cerevisiae.
- 31. The cell of claim 27 which is a bacterium.
- 32. A bacterial cell comprising the composition of claim 7.
- 33. A cell comprising the vector of claim 1 or 18.
- 34. The cell of claim 33 which is a bacterium.
- 35. A method of producing a vector containing a target nucleic acid, comprising the step of contacting under conditions which allow homologous recombination:
  - (a) a target nucleic acid;
  - (b) a first arm comprising a nucleic acid homologous to the 5' terminus of said target nucleic acid, a first selectable marker and a first cyclization element; and
  - (c) a second arm comprising a second nucleic acid homologous to the 3' terminus sequence of said target nucleic acid, a second selectable marker, and a second cyclization element,

wherein homologous recombination of (a), (b) and (c) produces said vector containing said target nucleic acid.



- (7) a nucleic acid homologous to the 5' terminus of said target nucleic acid;
- (8) a nucleic acid homologous to the 3' terminus of said target nucleic acid; and
- (9) at least one rare restriction endonuclease recognition site;

in a yeast cell wherein homologous recombination of (i) and (ii) produces said recombinant nucleic acid;

- (b) isolating said recombinant nucleic acid from said yeast cell; and
- (c) introducing said recombinant nucleic acid into a bacterium, wherein said recombinant nucleic acid is amplified in said bacterium.
- 45. The method of claim 44, wherein said yeast is Saccharomyces cerevisiae.
- 46. The method of claim 44, wherein said bacterium is Escherichia coli.
- 47. The method of claim 44, wherein said target nucleic acid comprises a virus nucleic acid.